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Renin-angiotensin system in neonatal rats: Induction of a renal abnormality in response to ACE inhibition or angiotensin II antagonism

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Renin-angiotensin system in neonatal rats: Induction of a renal abnormality in response to ACE inhibition or angiotensin II antagonism. In experiments designed to analyze cardiovascular structure in response to antihypertensive therapy with an ACE inhibitor, we decided to start very early in life with the aim to prevent blood pressure increases and the development of vascular structural changes. In these treated groups of rats we unexpectedly observed that after they were weaned, their water consumption and urine volume, respectively, increased substantially. The present study was designed to determine if inhibition of the renin-angiotensin system produced similar effects in different strains of rats, and focused on characterizing the abnormal fluid balance occurring as a consequence to neonatal treatment with ACE inhibitors or angiotensin II blockers. Three-day-old Wistar Kyoto (WKY), Wistar (WR) and spontaneously hypertensive rats (SHR) were given either saline, enalapril, captopril, losartan and the AT_2 blocker, PD123319, in the same amount of volume for 20 days. Treatment was stopped and rats were examined with regard to renal morphology at 4, 14 and 30 weeks of age. In addition, water consumption, urine volume, urine electrolytes and osmolality were analyzed at 14 weeks of age, that is, 10 weeks off treatment. Early treatment with the ACE inhibitors, enalapril and captopril, and the AT_1 blocker, losartan, but not the AT_2 blocker, PD 123319, in the SHR and in the normotensive strains WKY and WR produced persistent, irreversible histopathological renal abnormalities in adult life, long after the rats had been taken off treatment. These abnormalities consisted of mainly cortical tubulointerstitial inflammation, various degrees of papillary atrophy and pelvic dilation. These structural renal abnormalities impaired the urine concentrating ability in the treated animals, as evidenced by a reduced urine osmolality, and caused increases in water consumption and diuresis. These results suggest an important role for angiotensin II in the developing kidney during the first postnatal weeks or even days in the development of normal renal function, a situation that should be seriously considered in clinical situations when any extended ACE inhibitor therapy in newborns is discussed.

Angiotensin converting enzyme (ACE) inhibitors have been very effective in both reducing blood pressure and normalizing vascular structural changes in experimentally hypertensive rats [1–3] as well as altering vascular structure in normotensive animals [4]. Further, ACE inhibitors have been demonstrated to have persistent effects on vascular structure and blood pressure following treatment withdrawal in the spontaneously hypertensive rat (SHR) [1–3, 5]. In the latter studies [2, 3, 5], enalapril and ramipril, respectively, were given as monotherapy to both young and adult SHR, and regardless of when therapy was initiated, persistent "structural down-regulation" of heart and blood vessels was still evident long after cessation of treatment. In most studies, antihypertensive treatments in SHR were not started until the age of four to five weeks, focusing more on regression than on prevention of vascular changes.

A recent study by Adams, Bobik and Friberg [6] showed that there is a marked vascular structure-based increase in resistance even in two-week-old SHR compared with age- and body weight-matched Wistar Kyoto (WKY) normotensive rats. In studies aimed at preventing these very early structural changes measured in the hind limb skeletal muscle vascular bed of these neonatal SHR [6], a series of experiments in which an ACE inhibitor was given to neonatal SHR and WKY was initiated. After cessation of this early therapy, we observed, unexpectedly, a persistent and substantial increase in diuresis and consequently in water consumption in the post-weaning period [4]. Circumstantial evidence (*vide infra*) would attribute these effects to ACE inhibition/Angiotensin II antagonism.

The renin-angiotensin system is widely acknowledged to play an important role for blood pressure regulation and sodium and water balance in adult life. Less studied, however, is the functional role of the renin-angiotensin system during the fetal and the newborn periods. A physiological significance for this system has been proposed during maturation, but this influence seems to be more powerful near end-gestation and postnatal than in early gestation [7].

Enalapril has been shown to be fetotoxic when administered in late gestation in the rabbit, whereas up to mid gestation no effects on fetal wastage were observed [8]. Moreover, Schubiger, Flury and Nussberger [9] reported acute renal failure in a

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neonate exposed to ACE inhibition treatment during pregnancy. The background of this fetotoxicity and renal disorder is not known, but could be related to ACE inhibitor-induced changes in renal morphology with consequences for tubular concentrating mechanisms. Thus, ACE inhibitor treatment during these conditions may not necessarily be toxic *per se*, but the lack of angiotensin II (Ang II) receptor stimulation may induce a "physiologic" toxicity, which may secondarily impair renal function. In such a case, Ang II antagonism would result in similar renal damage, since the AT_1 receptor distribution is identical in the fetus to that found in the adult animal, while AT_2 receptors are abundant in the mesenchyme and no binding is evident in the kidney [10]. Similarly, in a very recent study by Tufro-McReddie et al [11], it was shown that kidney AT_1 mRNA levels increased from end-gestation to the newborn period and that renal AT_1 mRNA shifted location during maturation. Hence, there is clear cut evidence of existing Ang II receptors, predominantly AT_1 receptors, in the immature kidney, thus constituting possible action of ACE inhibitors and Ang II antagonists.

In the present study, we examined the effects of specific interventions in the activities of the renal angiotensin system on renal morphology, water consumption, urine volumes and electrolytes in SHR and normotensive control rats. We used two ACE inhibitors with different chemical properties: captopril and enalapril of various doses. In addition, to link the renal pathophysiological effects to lack of Ang II receptor stimulation, neonates were treated with losartan, an AT_1 receptor antagonist, and to elucidate the involvement of possible AT_2 receptors within the kidney as a possible cause for the fluid balance changes, the AT_2 receptor blocker PD123319 was also administered.

Methods

Animals

Several groups of male SHR, WKY and ordinary Wistar rats were used. Rats from colonies bred at the Baker Medical Research Institute (SHR stock originally supplied by Prof Y. Yamori in 1986), ALAB Breeding Centre (Södertälje, Sweden, mothers with pups aged 2 days) and Möllegaards Breeding Centre (Skensved, Denmark) were used. All rats were housed in rooms in which temperature was controlled around 25°C and a 12-hour light/dark cycle was maintained. Food and water were supplied *ad libitum*. Pregnant rats were observed carefully at end-gestation to determine the exact birth date for the pups. On the second day after birth, the mother was carefully handled and the pups were sexed; only males were subsequently injected in order to obtain similar adult body weights to correspond with the male groups that were examined for vascular structure design. Intraperitoneal daily injections started on the third day after birth. Body weights were obtained daily to give the correct dosing.

Group 1. Male SHR and WKY from the Baker Institute colony and WR from Möllegaard Breeding Centre, respectively, were injected with either enalapril maleate (10 mg/kg) or saline in standardized volumes of 10 μl/g. Injections started at the third day of age and continued until 22 days of age. These groups were then analyzed for renal histology at approximately four weeks of age by immersion fixation in 10% formaldehyde solution.

Group 2. Litters of male SHR and WKY from the Baker Institute colony were treated with enalapril according to the same procedure as used for group 1, but were then left untreated for 10 weeks. Body weight and water consumption were followed weekly. At 14 weeks of age, kidneys were weighed and extirpated for assessment of renal histology.

Group 3. In separate groups of male Wistar rats (ALAB), the AT_1 antagonist losartan (former DuP 753, DuPont-Merck, Wilmington, Delaware, USA) was used. Here neonates were injected i.p. with saline, 10 and 30 mg/kg of losartan and enalapril 10 mg/kg, using the same volume of injectate as in the other groups. Animals were given injections from day 3 until day 22, and were then taken off therapy and left untreated until 14 weeks of age.

Group 4. Three-day-old male Wistar rats were given 10 mg/kg of the AT_2 antagonist PD123319 (Park-Davis, USA) i.p. for 19 days. The rats were then taken off therapy and left untreated for 10 weeks. Wistar rats receiving 10 mg/kg of losartan were injected in parallel according to the same protocol.

Group 5. WKY rats from the Baker Institute colony were treated with enalapril from day 3 until day 22, and were then left untreated until 30 weeks of age, when kidneys were extirpated and assessed histopathologically. Age-matched saline-injected rats served as controls.

Group 6. WKY rats from the Baker Institute colony were also given captopril in two concentrations: 20 and 40 mg/kg, respectively. The volume of the injectate was unchanged compared with the enalapril regimen. Kidneys were extirpated at 4 (20 and 40 mg/kg) and 14 (20 mg/kg) weeks of age and subsequently examined histopathologically.

Fluid balance measurements

Water consumption was measured in rats from group 2 by means of weighing water bottles. In all other groups water consumption and urine volume measurements were assessed by means of metabolic cages. Rats were put in cages for at least two occasions, each period consisting of three days. They had free access to food and water. Values were then averaged for every 24 hours. However, the first 24 hour measurements were discarded. Urine was collected and frozen for subsequent analyses of osmolality, sodium and potassium.

Renal histology—Semiquantitative measurements

The examined kidney specimens were immersion-fixed. These were then imbedded in paraffin and sliced in a microtome for subsequent assessment by light microscopy. Several different histopathological features were determined: the degree of papillary atrophy, dilation of renal pelvis, glomerular sclerosis and tubulointerstitial (TI) inflammation. All kidneys were characterized using semi-quantitative assessments and each of the above-mentioned parameters were given a score on a graded scale, which consisted of 0 (no evident abnormalities), ±, +, ++ and +++. For calculation purposes, these symbols were expressed as ± = 1 and + = 2. The tubulointerstitial inflammation consisted mainly of infiltration of lymphocytes in the interstitium. In some kidneys infiltrate of granulocytes was also present, and in such cases it was found around tubular cells. In addition, granulocytes were also found to infiltrate the tubular

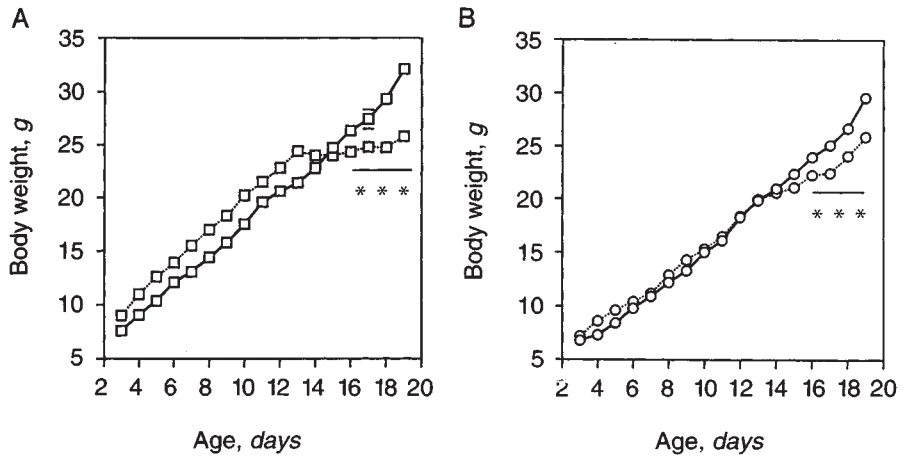

Fig. 1. *Body weights (g) of neonatal, male Wistar Kyoto rats (WKY, A, open squares) and spontaneously hypertensive rats (SHR, B, open circles) (groups 1 and 2) during daily intraperitoneal injections of enalapril 10 mg/kg (N = 19 for WKY and N = 28 for SHR, dotted lines) and saline (N = 14 for WKY and N = 18 for SHR, solid lines).* Note the notch in the body growth curve at day 13 of age for both SHR and WKY. From day 14 in both treated groups body weight is significantly reduced vs. saline control ($F = 49$, $P < 0.0001$, ANOVA). Solid lines indicate saline treatment and dotted lines indicate enalapril treatment. Data are mean ± SE. *** $P < 0.001$ versus respective saline group.

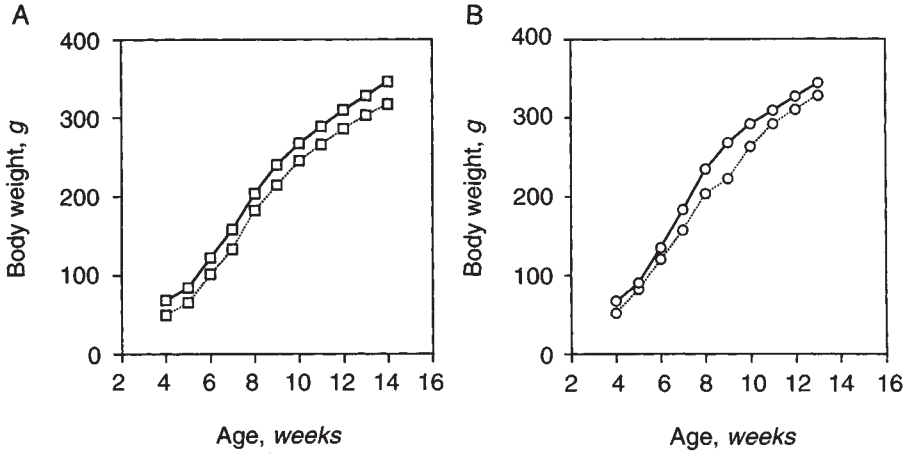

Fig. 2. *Body weight (g) curves from 4 to 14 weeks of age in early treated (N = 12 for WKY, A and N = 12 for SHR, B) WKY and their respective control, (solid lines) saline group (N = 14 for WKY and N = 17 for SHR) (group 2).* Both WKY and SHR treated with enalapril (dotted lines) remained at a lower body weight throughout the study period ($F = 178$, $P < 0.0001$, ANOVA). Symbols and lines as in Fig. 1. Data are mean ± SEM.

epithelium. All histopathological assessments were coded and were done by the researcher blind to the treatment.

Analyses of urine osmolality and electrolytes

Sodium and potassium samples of about 100 μl were measured in a flame spectrophotometer (FLM3, Radiometer, Copenhagen, Denmark) and for measurements of osmolality (20 μl sample) the method of freezing point depression was used (Wide Range Advanced Osmometer model 3MO, Advanced Instruments Inc., Needham Heights, Massachusetts, USA).

Statistical evaluation

Three-way ANOVA and Bonferroni's *post hoc* test were performed in testing for differences regarding body weights, water intake, osmolality. In addition, parametric (blood pressure responses to angiotensin) and non-parametric (renal morphology) tests of paired and unpaired design were used. Values are expressed as mean ± SEM. A P-value less than 0.05 was considered statistically significant.

Results

Body weights

Figure 1 shows the time course of increases in body weights when neonatal rats were treated with enalapril (ANOVA for treatment effect; $F = 120$). Enalapril treatment induced a delayed effect on the growth of the WKY (Fig. 1A) which became apparent about 13 days after initiating treatment when the rats were aged between 14 and 16 days. Body weight, although maintained, fell below those of the age-matched saline-injected WKY (Fig. 1B). A similar effect was observed in the neonatal SHR treated with enalapril. However, in this study the effect on body weight was not as great as in WKY (Fig. 1B).

Figure 2 shows the time course of increases in body weights from 4 to 14 weeks of age, that is, approximately from the cessation of neonatal treatment and 10 weeks off treatment. Due to the initial enalapril treatment a consistent reduction of body weight persisted throughout the whole study period, although it was not more than 9% (ANOVA for treatment effect, $F = 178$, $P < 0.0001$). Body weight was reduced to a similar degree in both WKY and SHR. Not only did treatment with the ACE inhibitor enalapril cause retardation of body weight, but also treatment with losartan resulted in a reduction of the normal body weight gain in young and adult ages compared with age-matched animals that received saline injections, similar to WKY (Figs. 1 and 2). Enalapril caused the most marked effect, while the effect of losartan was moderate. In addition, there was no difference in body weights when the losartan dose was increased from 10 to 30 mg/kg. There was no effect on body weight by PD123319 treatment.

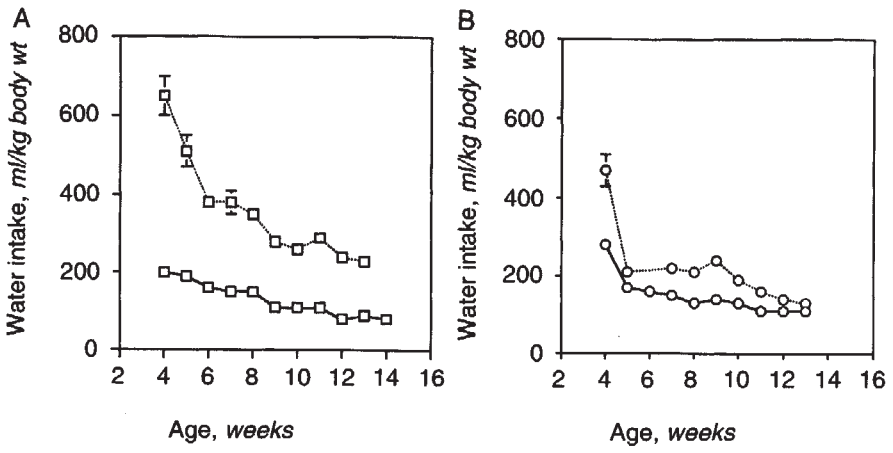

Fig. 3. *Water consumption curves (ml/kg body wt per 24 hr) from 4 to 14 weeks of age in WKY (A) and SHR (B) (group 2) treated neonatally with either enalapril (dotted lines) or saline (solid lines).* Symbols are as in Fig. 2; numbers of animals equal those given in Fig. 2. Note the dramatic treatment effect of enalapril in both groups ($F = 1141$, $P < 0.0001$, ANOVA). In addition, there is a marked difference between treated SHR and WKY, showing less effect of treatment in SHR ($F = 392$, $P < 0.0001$). Mean ± SEM is expressed in all data points.

Table 1. Fluid balance, sodium and potassium excretion and urine osmolality in male neonatally treated and untreated Wistar rats measured at 14 weeks of age

Treatment	N	Water intake	Urine volume	Na^+ excretion	K^+ excretion	Osmolality
		ml/kg/24 hr		*mmol/24 hr*		*mOsm/kg*
Saline	5	106 ± 12	46 ± 7	1.97 ± 0.05	2.65 ± 0.19	922 ± 185
Enalapril (10)	5	317 ± 25[a]	217 ± 23[a]	1.83 ± 0.18	2.94 ± 0.15	355 ± 22[a]
Losartan (10)	10	197 ± 20[a]	126 ± 13[a]	2.07 ± 0.14	2.83 ± 0.14	522 ± 49[a]
Losartan (30)	6	250 ± 23[a]	184 ± 21[a]	2.12 ± 0.14	3.14 ± 0.14	486 ± 54[a]
PD123319 (10)	7	71 ± 3	32 ± 4	1.64 ± 0.12	3.28 ± 0.27	1674 ± 138
Losartan (10)	6	174 ± 8[a]	119 ± 9[a]	1.76 ± 0.08	3.51 ± 0.16	308 ± 80[a]

Data are mean ± SE. Numbers in brackets represent dose in mg/kg.
[a] $P < 0.001$ between saline injected group and intervention groups and between the PD123319 and losartan, respectively

Table 2. Mean arterial pressure (MAP) responses following an acute injection of 50 ng Ang I in 14-week-old Wistar rats (group 3) exposed to neonatal treatment with either enalapril or saline

Treatment	MAP before	MAP after	Difference	SED	P<
	mm Hg				
Saline ($N = 8$)	113	142	29	4	0.001
Enalapril (10 mg/kg) ($N = 4$)	107	132	25	3	0.001

Data are means. SED is standard error of the difference.

Fluid balance and electrolytes

Both WKY, WR and SHR exposed to early treatment with enalapril and losartan respectively, showed a marked increase in water consumption and urine volume from four weeks until adult age, in spite of treatment withdrawal at four weeks of age (Fig. 3, Table 1). SHR in this study seemed to be less sensitive to the early therapy with enalapril with respect to water intake, which was similar to the situation for body weight. Treatment with PD123319 did not influence fluid balance at all; water intake and urinary output were similar to control values (Table 1). Urine excretion of sodium and potassium remained unaltered in all treated groups compared with control groups (Table 1). Urine osmolality was normal in the PD123319 treated group compared with saline controls, while in enalapril and losartan (10 and 30 mg/kg) treated animals, it was drastically reduced (Table 1). This indicates that the urine concentration ability was reduced markedly in rats that had been exposed to early postnatal, short-term inhibition of ACE or to AT_1 receptor blockade, respectively.

Blood pressure responses to angiotensin I

In group 3 rats receiving enalapril, the function of the RAS was assessed in adult animals treated neonatally by means of injecting Ang I and monitoring the MAP responses. Table 2 shows that these WR showed a similar rise in MAP, compared with saline treated rats, with no significant difference between groups, indicating at least a partially responsive renin-angiotensin system in the early enalapril-treated group.

Semiquantitative assessment of changes in renal histology

Rats that had been exposed neonatally to enalapril and losartan showed gross renal histopathological changes at both 4 and 14 weeks of age compared with controls (Fig. 4, Table 3). These consisted of tubulointerstitial (TI) chronic inflammation, various degrees of papillary atrophy and renal pelvic dilation. However, no kidneys showed any sign of glomerular sclerosis, indicating absence of chronic renal disease affecting glomeruli. In four-week-old neonatally treated SHR, WKY and WR, the degree of TI inflammation as determined by semiquantitative assessment was similar and less pronounced than at 14 weeks of age (Fig. 5A). In similarly treated WKY and WR examined 10 weeks after treatment, the TI inflammation score had progressed significantly (Fig. 5B), whereas it remained unaltered in similarly treated 14-week-old SHR (Fig. 5). In group 5 early treated WKY rats left untreated between 4 and 30 weeks of age,

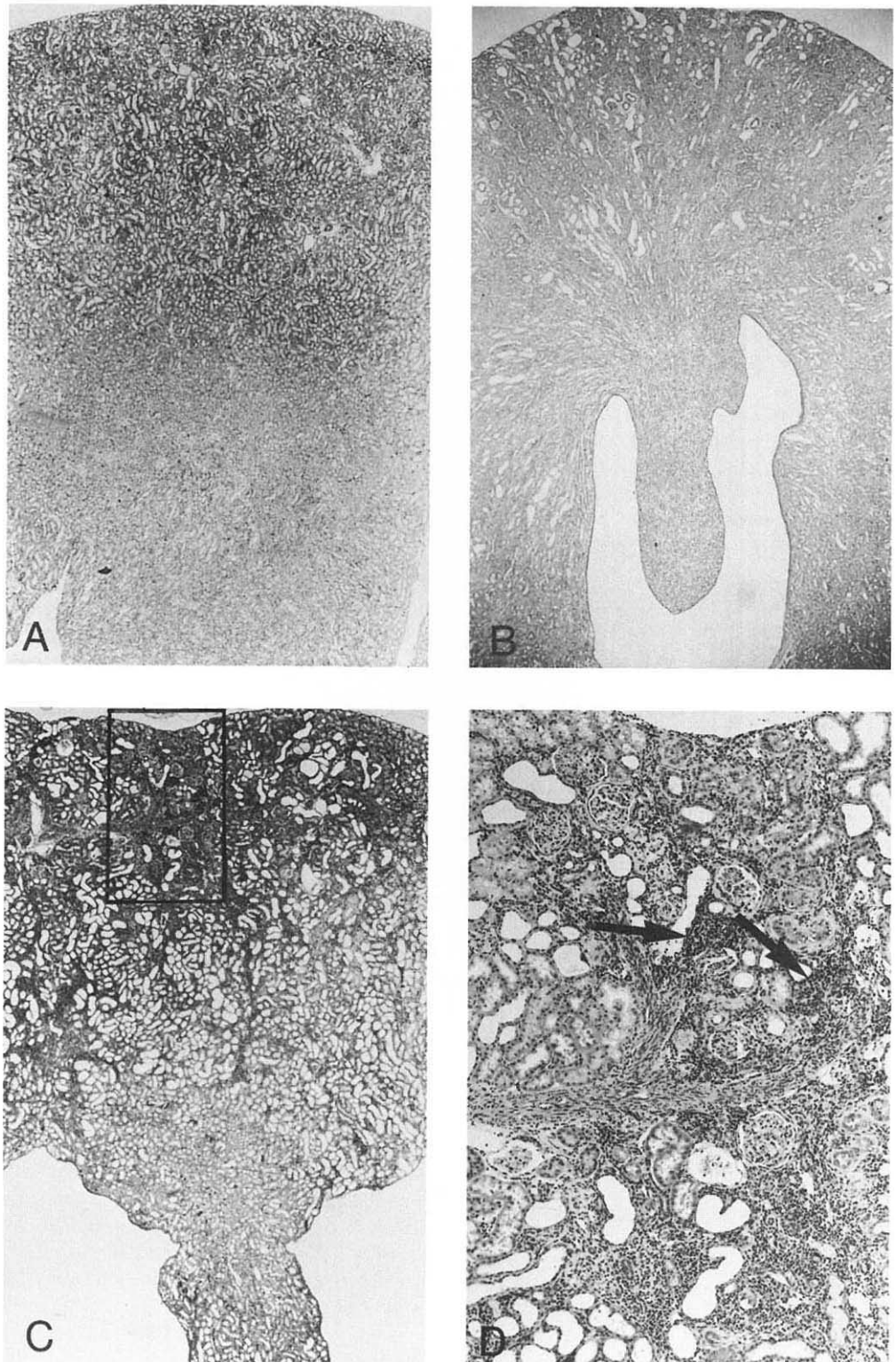

Fig. 4. *Kidney sections, light microscopy (hematoxylin and eosin).* **A.** WKY rat 14-weeks-old, control (magnification × 14). **B.** WR rat 4-weeks-old, enalapril treatment i.p. days 3 to 22. Note the papillary atrophy (magnification × 10.5). **C.** WKY rat 14-weeks-old, enalapril treatment days 3 to 22. The papillary atrophy and the tubulointerstitial inflammation are more pronounced than in B (magnification × 18). **D.** Higher magnification of marked area in C. Arrows show clusters of inflammatory cells in the tubulointerstitial tissue (magnification × 70).

the TI inflammation score further increased compared with the score at 14 weeks of age (from 6.50 ± 0.65 to 8.33 ± 2.02, $P < 0.05$, respectively). Thirty-week-old saline injected controls showed total lack of any pathological changes. Furthermore, a clear dose-dependent effect on TI inflammation score was evident in the groups receiving losartan 10 and 30 mg/kg (Fig. 5B).

Table 3. Semiquantative measurements of papillary atrophy and pelvic dilation in treated and untreated spontaneously hypertensive rats (SHR) and normotensive Wistar Kyoto rats (WKY) and ordinary Wistar rats (WR) of 4, 14 and 30 weeks of age

Group rats treatment	*N*	Age	Papillary atrophy score	Pelvic dilation score
1.WKY+SHR-saline	6	4	0	0
1.WKY+SHR-enalapril	4[c]	4	0	0
1.WR-enalapril *10 mg/kg*	20	4	2.10 ± 0.40[a]	3.60 ± 0.40[a]
2.WKY+SHR-saline	11	14	0	0
2.SHR-enalapril *10 mg/kg*	4	14	1.75 ± 0.48[a]	0.75 ± 0.48[a]
2.WKY-enalapril *10 mg/kg*	6	14	2.00 ± 0.00[a]	3.00 ± 0.73[a]
3.WR-saline	10	14	0	0.80 ± 0.61
3.WR-enalapril *10 mg/kg*	5	14	4.80 ± 0.37	2.80 ± 0.97
3.WR-losartan *10 mg/kg*	10	14	2.60 ± 0.54	3.10 ± 0.82
3.WR-losartan *30 mg/kg*	6	14	5.00 ± 0.82	0
4.WR-PD123319 *10 mg/kg*	7	14	0	0.43 ± 0.20
4.WR-losartan *10 mg/kg*	6	14	3.33 ± 0.45[b]	3.50 ± 0.70[b]
4.WR-saline	8	14	0	0
5.WKY-saline	7	30	0	0
6.WKY-enalapril *10 mg/kg*	5	30	2.40 ± 0.40[a]	0

Data are mean ± SE. The different groups are described in detail under **Methods**.

[a] Statistical significant difference, $P < 0.001$

[b] Statistical significant difference ($P < 0.001$) between losartan in group 4 and the other two groups in group 4

[c] Several specimens were not possible to analyze due to autolysis

A separate group of WKY (group 6) was given captopril neonatally. The renal histological examination revealed similar pathological changes as were induced by enalapril (compare to Fig. 4). Similar to the losartan groups, a dose-dependent effect with respect to TI inflammation score at four weeks of age could be observed (20 and 40 mg/kg of captopril, respectively, Fig. 6). One subgroup of WKY on early therapy with captopril (20 mg/kg) were left untreated from 4 to 14 weeks of age and the TI inflammation score progressed significantly compared with that obtained at four weeks of age (from 2.75 ± 0.25 to 8.00 ± 0.71, $P < 0.001$; Fig. 6).

A positive relationship was found between renal structure and function which is evident in Figure 7. It shows a significant correlation between TI score, that is, the degree of tubulointerstitial chronic inflammation and water intake, demonstrating the importance of intact renal morphology for maintaining a normal tubular concentrating ability.

A statistically significant degree of papillary atrophy was already evident at four weeks of age in early enalapril-treated WR, which also became evident in 14-week-old SHR and WKY (Table 3). None of the saline groups showed any sign of papillary atrophy. Similarly, both losartan regimens (10 and 30 mg/kg) caused papillary atrophy in a dose-dependent pattern. PD123319 treated animals showed no evidence of altered renal morphology or fluid balance (Table 3). Furthermore, we were not able to detect any sign of pelvic dilation in our limited number of saline-injected control rats, although this has been reported in Wistar rats [12, 13]. In the treated groups, the pelvic dilation score mimicked the pattern for papillary atrophy score, with the exception of the 30-week-old early enalapril-treated WKY, which for some reason showed papillary atrophy without concomitant pelvic dilation (Table 3).

Discussion

The main findings of the present study with respect to effects on renal structure and function following treatment of postnatal rats with either captopril/enalapril or losartan were: (i) reduced urine osmolality; (ii) increased urine production and consequently increased water consumption; (iii) gross morphological changes in the kidney. Normal fluid balance and normal renal morphology were, however, seen in animals treated with the AT_2 receptor antagonist PD123319. All treated groups showed normal urinary excretion of sodium and potassium. The alterations of fluid balance in the ACE inhibitor losartan treated groups were associated with marked cortical TI chronic inflammation but also to papillary atrophy and pelvic dilatation. Interestingly, urine volume and water intake were less affected by enalapril treatment in SHR at 14 weeks of age compared with similarly treated WKY, and accordingly the renal histopathological changes were less pronounced. A progression of the histopathological changes were noted between 4 and 14 weeks of age in WKY, which was even further exaggerated between 14 and 30 weeks of age, whereas no progression was evident in 14-week-old SHR versus 4-week-old SHR. These findings indicate that an intact renin-angiotensin system is needed in early life for development of a normal tubular concentrating ability. This effect seems to be specifically related to a lack of stimulation by renal AT_1 receptors.

Renal AT_1 receptors, determined either by receptor binding assay or gene expression, have been demonstrated in newborn rats which showed the same pattern as in adult rats [9, 10]. Evidence for the presence of AT_1 receptors has been presented in glomeruli, proximal tubule, and inner stripe of the outer medulla [14]. However, recent data by Zhuo et al [15], using high resolution light and electron microscopic autoradiography, demonstrated that type 1 interstitial cells located in the inner stripe of the outer medulla are the primary sites for Ang II receptors. A lack of AT_1 receptor stimulation may, by disturbing the intrarenal Ang II levels and the subsequent interaction with type 1 interstitial cells, lead to an altered medullary microcirculation and/or tubular function.

The significance of the renin-angiotensin system during the late fetal stage and in the newborn period has been recognized [7, 16]. It is therefore tempting to speculate that the lack of stimulation of Ang II receptors at this early time in life leads to tubular inflammation, which subsequently spreads to the interstitium, and finally creates papillary atrophy and pelvic dilation. Regardless of the mechanism, it is clear that the presence of local/circulating Ang II during early development is critical for maintenance of renal functional and structural integrity.

It is reasonable to believe that the neonatal treatment-induced renal damage was not due to a completely nonfunctioning renin-angiotensin system in adult age, since injection of a small dose of Ang I resulted in marked pressure elevation. The pressure response to Ang I did not differ from saline-injected controls. Thus, in all likelihood, the tissue ACE should also be preserved, which favors the hypothesis that the renal damage secondarily caused the fluid balance changes. One could argue, however, that the early treatment with enalapril/captopril caused irreversible CNS changes within the regions

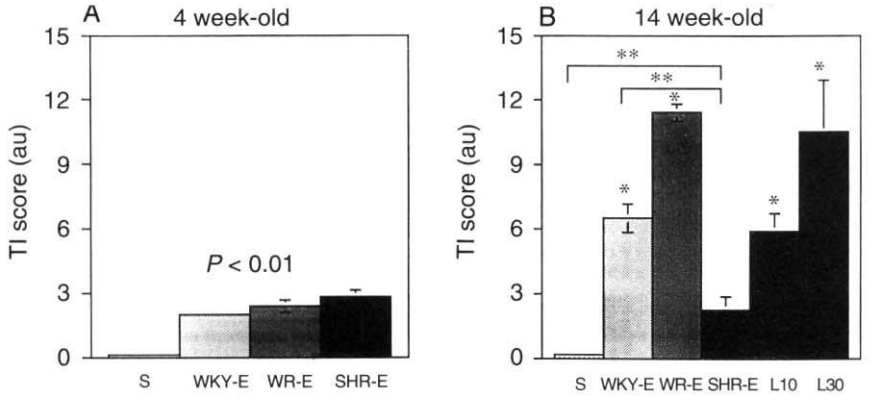

Fig. 5. *Chronic tubulointerstitial inflammation (TI score, arbitrary units) in 4-(A, group 1) and 14-(B, groups 2 and 3)-week-old WR, WKY and SHR treated neonatally with saline (S), enalapril 10 mg/kg (E), losartan 10 (L10) and 30 mg/kg (L30).* There was a clearcut progression of TI score during the 10 week treatment free interval. In addition, a dose-dependent effect on TI score was seen with losartan therapy. * $P < 0.001$ from saline-injected rats, ** $P < 0.01$.

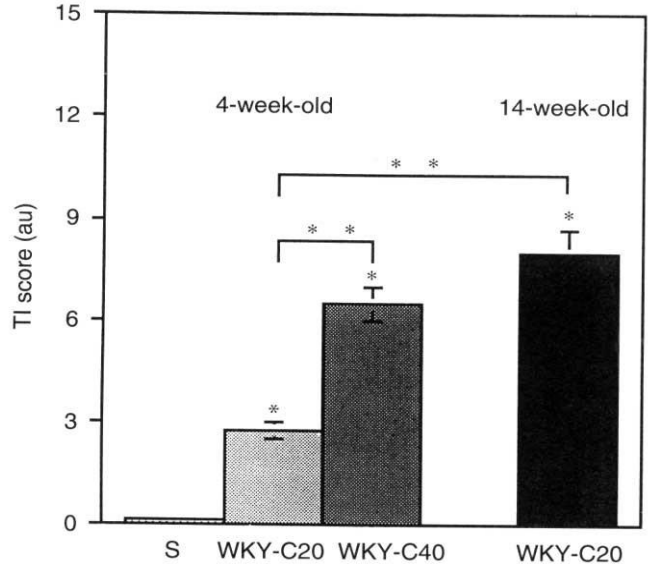

Fig. 6. *Chronic tubulointerstitial inflammation (TI score) in 4 and 14-week-old WKY rats exposed to neonatal treatment with either saline or 20 and 40 mg/kg captopril, respectively (group 6).* As with losartan 10 and 30 mg/kg treatment, also captopril demonstrated more renal damage with increasing dose. * $P < 0.001$ from saline-injected controls, ** $P < 0.01$.

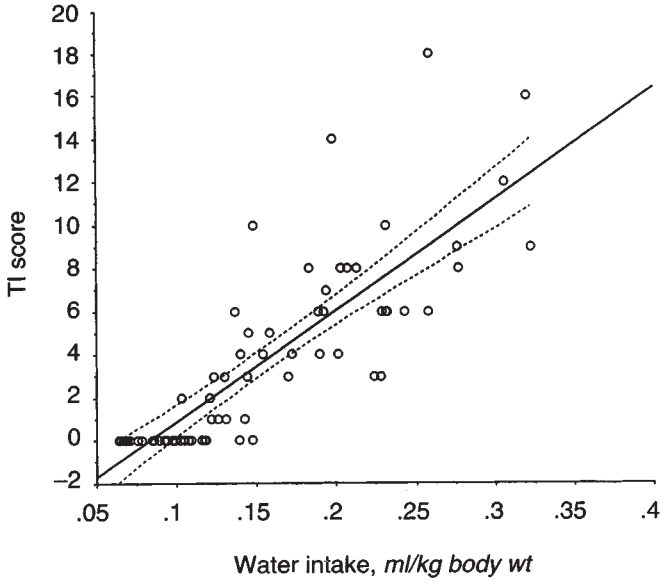

Fig. 7. *Linear regression between TI score and water consumption in neonatally enalapril and saline treated Wistar rats.* Water consumption was measured in metabolic cages at 14 weeks of age. Shortly thereafter the rats were sacrificed and renal morphology was assessed semiquantitatively. Dotted lines indicate 95% confidence interval of the absolute TI score value. $r = 0.828$ ($F = 142$, $P < 0.0001$, ANOVA).

governing thirst and salt appetite. This explanation is less likely since enalapril does not inhibit brain ACE in SHR following oral administration [17]. In addition, Brattleboro rats born with diabetes insipidus and a central polydipsia demonstrate a totally normal renal medullary morphology [18]. Taken together, the findings from these latter studies argue against any CNS-induced changes in fluid balance, and support the concept that the observed renal damage *per se* hampers tubular concentrating mechanisms.

Chronic interstitial inflammation has also been reported in other instances associated with potential impairment of the renin-angiotensin system. For example, chronic interstitial inflammation has been observed in marmosets and SHR and WKY which were actively immunized against renin [19, 20]. These studies, in contrast to the present one, began their therapy in older animals and the authors demonstrated immunoglobulins, mononuclear cell infiltration and fibrosis around the juxtaglomerular apparatus, while in the present study glomeruli and the juxtaglomerular apparatus were morphologically unchanged.

It can be speculated that during the first two weeks of ACE inhibitor treatment or AT_1 antagonism the kidney damage is likely to occur, since no kidney pathology was reported in adult SHR and WKY by, for example, Harrap and co-workers [3], who initiated ACE inhibitor therapy at two weeks of age, and Lee et al [21], who gave captopril to the mothers during both pregnancy and lactation. In the latter study it may be that very little drug was delivered to the pups via the placenta and breast milk, respectively. Those factors may interfere with the interpretation of study results, at least with regard to effective drug concentration in the pups during fetal and early postnatal periods. In our group we were not able to demonstrate any renal abnormalities in four-week-old rats that received enalapril via breast feeding despite the very high dose of 100 mg/kg enalapril

in drinking water to their mothers (Friberg and Adams, unpublished observation), suggesting that the effective dose in the neonate was not high enough to cause renal histopathological changes.

Already at four weeks of age there was clear papillary atrophy and signs of renal pelvic dilation in WR, which did not further progress to 14 weeks of age (Table 3). In contrast to TI inflammation score, we did not observe any progression of papillary atrophy or pelvic dilation scores in 30-week-old WKY. In the context of pelvic dilation (hydronephrosis) in rats, one should bear in mind that this phenomenon occurs spontaneously (in approximately 2% [12]), with secondary physiological consequences for renal hemodynamics and function [13]. At present we have examined a vast number of kidneys morphologically, and among them many control rat kidneys of various strains, and we have not been able to detect any sign of spontaneous, congenital hydronephrosis. This suggests that the various degree of pelvic dilatation and the associated TI inflammation observed in the present study was specifically linked to ACE inhibitor/AT_1 antagonism therapy.

In summary, we have observed that early treatment with ACE inhibitors (enalapril/captopril) and the AT_1 blocker losartan, but not the AT_2 blocker PD123319, in SHR and in normotensive strains WKY and WR produces persistent, irreversible histopathological renal abnormalities in adult life long after the rats have been taken off treatment, mainly consisting of cortical tubulointerstitial inflammation, various degrees of papillary atrophy and pelvic dilation. These structural renal abnormalities impaired the urine concentrating ability in the treated animals, as evidenced by a reduced urine osmolality, and caused marked diuresis and thirst. These results suggest an important role for intrarenal and/or circulating angiotensin II actions within the kidney during the first postnatal weeks or even days, in the development of normal renal function, and they should be considered in clinical situations when any extended ACE inhibitor therapy in newborns is discussed.

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